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IN RE APPLN. OF:	SOGABE ET AL.
PATENT APPLICATION NO.	09/940,941
FILED:	AUGUST 28, 2001
FOR:	CREATINE AMIDINOHYDROLASE, PRODUCTION THEREOF AND USE THEREOF

A confirmation copy of the transmitted document will:

☒ **Not be sent.**

In re Appln. of Sogabe et al.
Application No. 09/940,941

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In re Appln. of Sogabe et al.
Application No. 09/940,941

relied upon for an earlier filing date under 35 USC 120 in which copies of the references were previously furnished are set out below:

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Partial English translation of JP 62-91182 A

The physico-chemical characteristics of the creatine amidinohydrolase obtained in this invention is set forth in the following.

① Action

This enzyme hydrolyzes creatine to produce urea and sarcosine. The Km (Michaelis constant) value for creatine is 4.83×10^{-3} M (37℃, pH 7.8).

② Determination method of activity

To 50 mM phosphate buffer (pH 7.8, 1.0 ml) containing 0.1 M creatine is added a properly diluted enzyme solution (0.1 ml), and the mixture is reacted at 37℃ for 10 minutes. To the mixture is added a *p*-dimethyl aminobenzaldehyde solution (2.0 ml) (which is prepared by dissolving dimethyl aminobenzaldehyde (2.0 g) in dimethyl sulfoxide (100 ml) and adding conc. HCl (15 ml) thereto), and the mixture is stood at 25℃ for 20 minutes. The absorbance (△OD) at 435 nm is determined with a spectrophotometer using the sample at 0 minute of the enzyme reaction as a reference, and the enzyme activity is calculated as follows.

Enzyme activity unit per 1 ml of the enzyme solution (U/ml)=

$$\triangle OD \times 9.66 \times \text{enzyme dilution fold}$$

wherein the enzyme activity of creatine amidinohydrolase is defined that the enzyme amount that produces 1 μ mol of urea per minute under the above-mentioned conditions is one unit (1U).

③ Optimal pH

The optimal pH is near 7.0-8.0.

The buffers used are phosphate buffers (pH 6.0-8.0), Tris-HCl buffers (pH 7.0-9.0), and carbonate buffers (pH 8.5-9.5).

④ pH stability

An enzyme was added to buffers having various pH values, the mixture was incubated at 5℃ for 48 hours, and the residual enzyme activity was determined. The buffers used are the same as above. As a result, the creatine amidinohydrolase is found to be stable at near pH 7.0-8.5.

⑤ Heat stability

A solution (1.0 ml) of creatine amidinohydrolase in 50 mM phosphate buffer (pH 7.5) was treated at various temperatures for 30 minutes, and the residual activity of the enzyme was determined. As a result, the creatine amidinohydrolase is found to be stable at near 40℃ or below.

⑥ Optimal temperature

The optimal temperature for the reaction of the creatine amidinohydrolase is found to be near 40℃.

⑦ Molecular weight

About 50,000 (determined by gel filtration)